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THE CORRELATION BETWEEN CATALASE ENZYME ACTIVITY AND FERROPTOSIS IN PATIENTS WITH β-THALASSEMIA MAJOR AGED (5-15 YEARS)

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June 2022

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ABSTRACT

THE CORRELATION BETWEEN CATALASE ENZYME ACTIVITY AND FERROPTOSIS IN PATIENTS WITH β-THALASSEMIA MAJOR AGED (5-15 YEARS)

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Master of Science in Chemistry

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Worldwide, the thalassemia gene mutation is the most common. Patients with b-thalassemia major who have prolonged anemia and severe iron overload may benefit from blood transfusion therapy, particularly those who have undergone just blood transfusion therapy. The formation of lipid peroxidation products caused by iron-catalyzed oxidation of polyunsaturated fatty acids is known as ferroptosis, and it may be treated pharmacologically using iron chelators and lipid peroxidation inhibitors. This research comprised 150 male children with beta- thalassemia major. Malondialdehyde, hemoglobin, ferritin, catalase activity, ROS, protein-carbon group, total antioxidant capacity, total thiols, and glutathione peroxidase 4 levels were tested in the serum. The findings were compared to 150 healthy male youngsters (males). When compared to the control group, there was a significant increase in the levels of Malondialdehyde (3.7844), ferritin (3337.0±1475), hemoglobin (7.5 ±1.0), catalase activity (0.2716), ROS (5.5559), protein carbonyl group (1.3975), and a significant decrease in the levels of glutathione peroxidase 4 (5.3741), total antioxidant capacity (700.9567), and total thiols This suggests that oxidative stress and a reduction in the antioxidant defense system play a key role in the etiology of beta-thalassemia major.

2022, 36 pages

Keywords: Thalassemia, Ferroptosis, Catalase enzyme, Reactive oxygen species

ÖZET

β-TALAZEMİ MAJOR YAŞ (5-15 YAŞ) HASTALARINDA KATALAZ ENZİM AKTİVİTESİ İLE FERROPTOZ ARASINDAKİ İLİŞKİ

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Dünya çapında, talasemi gen mutasyonu en yaygın olanıdır. Uzun süreli anemisi ve aşırı demir yüklenmesi olan b-talasemi majörlü hastalar, özellikle sadece kan transfüzyon tedavisi görmüş olanlar, kan transfüzyon tedavisinden fayda görebilirler. Çoklu doymamış yağ asitlerinin demir katalizli oksidasyonunun neden olduğu lipid peroksidasyon ürünlerinin oluşumu ferroptoz olarak bilinir ve demir şelatörleri ve lipid peroksidasyon inhibitörleri kullanılarak farmakolojik olarak tedavi edilebilir. Bu araştırmaya beta talasemi majörlü 150 erkek çocuk dahil edildi. Serumda malondialdehit, hemoglobin, ferritin, katalaz aktivitesi, ROS, protein-karbon grubu, toplam antioksidan kapasite, toplam tiyoller ve glutatyon peroksidaz 4 seviyeleri test edildi. Bulgular 150 sağlıklı erkek gençle (erkekler) karşılaştırıldı. Kontrol grubu ile karşılaştırıldığında Malondialdehit (3.7844), ferritin (3337,0±1475), hemoglobin (7,5± 1,0), katalaz aktivitesi (0.2716), ROS (5.5559), protein karbonil grubu (1.3975) düzeylerinde anlamlı artış vardı. ve glutatyon peroksidaz 4 (5.3741), toplam antioksidan kapasite (700.9567) ve toplam tiyol düzeylerinde önemli bir azalma Bu, oksidatif stres ve antioksidan savunma sistemindeki azalmanın beta-talasemi etiyolojisinde anahtar rol oynadığını düşündürmektedir.

2022, 36 sayfa

Anahtar Kelimeler: Talasemi, Ferroptoz, Katalaz enzimi, Reaktif oksijen türleri

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LIST OF SYMBOLS

%	Percent
±	Plus-minus
°C	Degrees Celsius
dL	Deciliter
g	Gram
L	Liter
mg	Milligram
mL	Milliliters
ng	Nanogram
nm	Nanometre
nmol	Nanomoles
α	Alpha
β	Beta
γ	Gama

LIST OF ABBREVIATIONS

ALOX	Lipoxygenase
BIL	Bilirubin
CAT	Catalase
DNA	Deoxyribonucleic acid
GR	Glutathione reductase
GPx	Glutathione peroxidase
GPx4	Glutathione peroxidase 4
GSH	Glutathione
Hb	Hemoglobin
HBB	Hemoglobin subunit beta
HbF	Fetal hemoglobin
MEL	Melatonin
MDA	Malondialdehyde
mRNA	Messenger ribonucleic acid
NMD	Nonsense-mediated mRNA decay
Pas	Polyamines
PHD	Prolyl hydroxylases domain
Prxs	Peroxiredoxins
RAS	Renin–angiotensin system
RBC	Red blood cells
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TM	Thalassemia
UA	uric acid

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1. INTRODUCTION

One or more hemoglobin chains may be abnormally produced, causing the thalassemias, which are a genetic hemoglobin disorder. It is thought that α- and β-thalassemia are the most frequent forms of the blood disorder thalassemia. People with β-thalassemia are more likely to have deletional mutations than point mutations, which create the disease's characteristic chronic anemia in its victims. There are autosomal recessive genetic disorders that result from point changes in the β-globin gene and its surrounding regions. Traits, minor, intermediate, and major are used to describe thalassemia's severity. In contrast to the severe symptoms and frequent blood transfusions experienced by someone with thalassemia major, those without symptoms or with just mild to moderate anemia may have thalassemia trait. A controlled necrotic cell death, ferroptosis differs from other known cell death mechanisms in that it has distinct metabolic limits. Iron-dependent lipid peroxidation damages the cell membrane, causing phospholipid oxidation and membrane rupture, which ultimately leads in cell death. oxidative stress may have a role in the development of a broad spectrum of noncommunicable illnesses, according to increasing evidence (chronic diseases). Oxygen atoms are one of two kinds of reacting molecules (ROS). Chemical entities with one or more electron densities known as "free radicals" are found in both types of reactive species. When cells, tissues, and the extracellular matrix are exposed to free radicals, several internal defense systems (enzymatic and non-enzymatic) go into overdrive to remove the radicals and their byproducts. GSH, UA, MEL, BIL, and polyamines are examples of non-enzymatic antioxidants that may swiftly neutralize free radicals and oxidants (PAs). The oxidative enzyme superoxide dismutase is critical in the body's first line of defense (SOD). Disproportionation of superoxygen anion to hydrogen peroxide and molecular oxygen is catalyzed by the enzyme superoxygen dismutase (SOD). Additionally, the first line of defense enzymatic antioxidants include CAT, GPx, GSH-reducing enzymes (GR), and peroxiredoxins (Prxs) Hydrogen peroxide and these enzymes (catalase glutathione peroxidase) react to produce water and oxygen molecules (catalase).

1.1 Objectives of Study

Catalase enzyme activity and ferroptosis in β-thalassemia major patients of all ages will be examined in this research (5-15 years)

2. LITERATURE REVIEW

2.1 Thalassemia

It is estimated that at least 60,000 individuals are born each year with thalassemia, one of the most common inherited illnesses. Individuals from tropical and subtropical climates are more likely to possess such a mutation. Thalassemia mutation produces a variety of problems, including iron excess, bone abnormalities, and cardiovascular disease (Taher *et al.* 2018). Diseases that are caused by a reduction in the synthesis of hemoglobin's alpha or beta chains are a significant hereditary disorder (Hb). A component of the red blood cell, hemoglobin, carries oxygen. The majority of the molecule is made up of beta and alpha proteins. One or both of these proteins are insufficiently produced in the body, leading to abnormally formed red blood cells that are unable to carry adequate oxygen throughout the body. Anemia may begin as early as infancy and persist throughout life. Because thalassemia is a genetic disorder, at least one of the parents must carry the disease. Either a genetic mutation or the deletion of certain, crucial gene regions is to blame for this condition (Shafi *et al.* 2020). Hemoglobin, the material required for oxygen delivery, accounts for about 95% of the dry weight of a red blood cell. Hemoglobin is a protein, a molecule consists of four polypeptide chains (a tetramer), each of which includes more than 140 amino acids (Coll-Satue *et al.* 2021). Hemoglobin is composed of four chains of connected protein molecules (globulins). For healthy adults, hemoglobin consists of one alpha-globulin chain and two beta-globulin chains (abbreviated Hgb or Hb). For the most part, beta chains are absent from the hemoglobin molecule in fetuses and neonates. The gamma chains are eventually replaced by beta chains, resulting in the adult hemoglobin structure as the child gets older (Sudha 2020). Each globulin chain contains heme, an iron-containing porphyrin molecule that is important for cellular function. The iron atom in the heme molecule is necessary for the transportation of oxygen and carbon dioxide in human blood (2-1). Iron in hemoglobin contributes to the blood's red hue (Pek 2019). Hemoglobin is also necessary for red blood cell structural preservation. Like a doughnut without a hole in the middle, red blood cells are naturally spherical with thin cores (Sadhvi and Suresh 2020). The incorrect hemoglobin structure may

cause red blood cells to be distorted in form, as shown in Figure 2.1, reducing their ability to function and flow through blood vessels. Consequently (Alzubaidi *et al.* 2020).

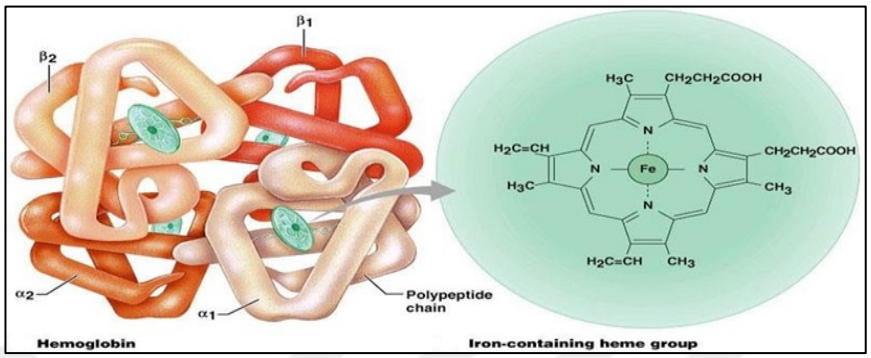

Figure 2.1 Structure of hemoglobin

2.1.1 Thalassemia classification

Thalassemia is divided into two types: alpha and beta thalassemia, which are inherited in an autosomal recessive way. Each of them is further classified into several categories based on the severity of the mutation (Jorde *et al.* 2019).

A - Alpha thalassemia is one of the most frequent genetic illnesses that results in hemoglobin abnormalities. It is typical in tropical and subtropical places where malaria is prevalent to have alpha thalassemia (Shang and Xu 2017). This form of thalassemia mutation reduces alpha-globin production, resulting in fewer alpha-globin chains generated, leading in an excess of 6 chains and an excess of 7 chains. The extra 6 chains combine to generate unstable tetramers known as Hemoglobin H (Kalle Kwaifa *et al.* 2020).

B - Blood illnesses that affect the synthesis of haemoglobin are known as beta-thalassemias, and they are characterized by a wide variety of phenotypes, from severe anemia to clinically unaffected carriers. The prevalence of beta thalassemia in the general population is estimated to be one in 100,000 individuals. Mutations in the HBB

gene on chromosome 11 are the primary cause of beta-thalassemia, which is passed down via the maternal line. The type of the mutation determines the severity of the condition (Sankar and Villa 2021).

2.1.2 Classification of beta thalassemia

1. Thalassemia minor carriers are usually unaffected, however they may have mild anemia at times. Every pregnancy in which both parents are carriers increases the risk of producing a child with inherited thalassemia by 25% (Bajwa and Basit, 2019).
2. Thalassemia intermedia is a less severe version of the disease that shows up later and causes less anemia. As a result, fewer blood transfusions are necessary. Oxidative stress is also a problem for people with this type (Taher *et al.* 2021).
3. One of the most frequent autosomal recessive diseases is beta thalassemia major. Throughout Africa, the Middle East, Central Asia, and the Indian subcontinent, it is prevalent. Thalassemia major is clinically obvious between the ages of six and twenty-four months. Growth retardation, pale complexion, jaundice, weakened muscles, and leg ulcers are some of the symptoms that patients with thalassemia major suffer from, as are extramedullary hematopoiesis and abnormalities in bone marrow growth. Bone abnormalities in the legs and prominent malar eminences are common, as is a tendency to have mongoloid slanted eyes and hypertrophy of the maxillary teeth, which allows for more visible upper teeth (Polat 2021). Medical treatment for this kind of thalassemia entails frequent lifetime blood transfusions, which may result in issues with the spleen. Patients are subjected to regular blood transfusions, which contribute to or worsen iron excess. Iron chelation therapy is required to protect internal organs. Because to advancements in iron chelation therapy, people with thalassemia major may now live extended lives with correct care. Untreated thalassemia major ultimately leads to mortality, most often from heart failure (Matar *et al.* 2021).

2.1.3 The genetic basis of Beta –thalassemia

For those who are carriers of the β0-thalassemia allele, no globin is produced, for those who are carriers of the β+-thalassemia, some β-globin is produced. In a formalized manner There are very few instances when deletions are the cause of a α-thalassemia. 290 bp to 60 kb deletions impact just the β-globin gene, which is the only one affected by this set of deletions. They all have the same deletion of 619 bp at the end of the gene. Other deletions, however rare, have functional and phenotypic significance because to their association with very high HbA2 levels in heterozygotes. They should be studied further. From the mRNA cap site, where the CACCC, CCAAT, and TATA elements are found, these deletions vary in size from –125 to +78 (Origa, 2017, Thein, 2018). The deletion of the gene's 5' promoter region seems to be the mechanism responsible for the much increased levels of HbA2 (Jiang *et al.* 2020). An enhanced interaction with the γ and δ genes in cis might lead to an increase in expression as a consequence of decreased competition for the upstream LCR (Jiang *et al.* 2021). Transposition of the δ -globin gene to deletion of the β-globin gene, there is a disproportionate rise in HbA2 (α2 δ2) (Zuo *et al.* 2021). It may also explain the slight increases in HbF that characterize this type of deletions, along with those induced by point mutations in the promoter region. In spite of heterozygotes' varied and modest increases in HbF, they are sufficient to make up for the homozygous lack of β -globin. Although the full lack of HbA2, two homozygotes for distinct mutations of this type exhibit a minor illness (α2 β2) (Greene *et al.* 2020). The large proportion of β-thalassemias are caused by point mutations in the gene or its surrounding areas. Transcription, RNA processing, and RNA translation are the methods by which single base changes, minor modifications, or deletions of a few bases affect gene regulation. The homologous DNA sequences that make up the β-globin promoter or the 50-nucleotide stretch in the 5'UTR may be altered by mutations that affect transcription. In general, they generate a small to moderate reduction in β-globin synthesis and may be undetectable in carriers (De Simone *et al.* 2020). Between codons 30 and 31 and 104 and 105, all β -globin–like genes contain three exons and two introns. There are about half as many -thalassemia alleles as -thalassemia alleles and in all cases, there is a lack of production of the beta-globin. Frameshifts and nonsense mutations are the most common source of these problems, and they nearly always occur

in exons 1 and 2 of the genome. NMD of the aberrant mRNA in erythroid cells is linked to early termination mutations (in exons 1 and 2) and low beta mRNA levels in steady-state erythroid cells (Borgatti *et al.* 2020).

2.2 Ferroptosis

Ferroptosis, a new kind of cell death, is distinguished by the buildup of iron and lipid peroxidation throughout the cell death process. Ironically, ferroptosis-inducing substances may directly or indirectly impact the antioxidant capacity of cells, leading in a rise in lipid reactive oxygen species (ROS), which may contribute to the oxidative cell death of ferroptotic cells. This condition is associated to several disorders, such as cancer, ischemia-reperfusion syndrome (IRS), renal impairment, and blood ailments (Li *et al.* 2020).

2.2.1 Morphological characteristics

Ferroptosis varies from apoptosis, necrosis, and autophagy in cell morphology, chemistry, and genetics, necrosis-like morphological abnormalities may be seen in cells that have undergone ferroptosis. Moderate chromatin condensation and cytoplasmic expansion are two of these characteristics. Cell separation and rounding, as well as an increase in autophagosomes, may occur in certain cases. When ferroptosis occurs in one cell, it may swiftly spread to other cells. Typical anomalies in mitochondria include condensing or swelling, increased membrane density, decreased or nonexistent crista, and rupture of the outer membrane of ferroptotic cells (Ma *et al.* 2021).

2.3 Biochemical Characteristics

2.3.1 Iron accumulation

Iron overload occurs when the body's iron levels are excess. Primary iron overload is often passed via families. Secondary iron overload is often caused by factors such as

transfusion, hemolysis, or excessive parenteral and/or dietary iron intake. Excess iron is collected in organs throughout the body, which may cause organ damage. The liver, heart, and endocrine glands are perhaps the most usually affected organs as a result of iron accumulation (Becker 2020). Erastin and RSL, the classic ferroptosis activators, block the antioxidant system increases intracellular iron storage. Through the Fenton reaction, iron may directly create excessive ROS, increasing oxidative damage. ALOX and PHD, enzymes involved in lipid peroxidation and oxygen balancing, may also be increased by iron. Ferroptosis susceptibility is influenced by alterations in iron control at the cellular and systemic levels. If iron overload genes are targeted or iron-chelating compounds are used, ferroptotic cell death may be successfully averted. For some reason, ferroptosis may be induced by iron alone, although the Fenton reaction produces ROS in other metals (such as zinc). After the creation of lipid ROS, iron excess may activate downstream effectors that aid in ferroptosis, according to one theory (Tang *et al.* 2020).

2.3.2 Lipid peroxidation

When the cell membrane is exposed to free radicals, the lipid peroxidation process occurs. There are two primary lipid hydroperoxides and reactive aldehydes generated during ferroptosis: MDA and 4-hydroxynonenal (4HNE). These fatty acids may be found in a variety of forms, including saturated, mono-unsaturated, and poly-unsaturated forms (PUFAs). However, peroxidation by ALOXs of PUFAs in phospholipids seems to be particularly important for ferroptosis, despite the fact that numerous cell membrane lipids may be oxidized (phosphatidylcholine, PE, and cardiolipin). Despite significant alterations in mitochondria, there is no evidence of cardiolipin peroxidation during ferroptosis (Aldrovandi *et al.* 2021).

2.3.3 Genetic features

Some genes or proteins, such as PTGS2/COX2, have been used as biomarkers for ferroptosis. Ferroptosis does not need prostaglandins as a substrate for lipid peroxidation. Acryl-CoA synthetase long chain member 4 (ACSL4), an enzyme

involved in the fatty acid metabolism of phospholipids, has been discovered as a biomarker and driver of ferroptosis in ferroptosis. Cells that lack the cellular ferroptosis regulator ACSL4 may nevertheless undergo ferroptosis in certain conditions. Ferroptosis-induced membrane damage is minimized by antioxidant defense genes (such as the glutathione and coenzyme Q10 (CoQ10) systems and the nuclear factor erythroid 2-like 2 (NFE2L2, also known as NRF2) transcription pathway) and membrane repair genes (such as the endosomal sorting complexes needed for transport (ESCRT)-III pathway) (Tang *et al.* 2021).

2.3.4 Immune characteristics

There are numerous intracellular signal transduction pathways in ferroptotic cells that can release and activate damage-associated molecular patterns (such as those found in HMB1 and DNA) as well as lipid oxidation products (such as those found in 4HNE, oxPLs, LTB4, LTC4, LTD4, or PGE2), all of which can result in a variety of immune and inflammatory reactions (Liu *et al.* 2021).

2.3.5 Mechanisms of ferroptosis

Ferroptosis pathways are activated. Glutathione (GSH) is lost and glutathione peroxidases 4 become inactive when system xc- is inhibited (GPX4). It is possible to synthesize glutathione (GSH) from methionine by using the transsulphuration pathway, which is hindered by cysteinyl tRNA synthetase. Ras-selective lethal small molecule 3 (RSL3) inhibits glutathione peroxidase 4 activity by covalent interaction with GPX4 (GPX4). Fat peroxides and ferroptosis may occur from the deactivation of GPX4. Ferroptosis is regulated by glutaminolysis enzymes (GLS2 and glutamic-oxaloacetic transaminase 1 (GOT1). The tricarboxylic acid cycle enhances cellular glutathione (GSH) loss and causes ferroptosis when mixed with cysteine deprivation. Ferroptosis is controlled by mitochondrial genes (ACSF2). ATF4-dependent CHAC 1 is produced in response to the Endoplasmic Reticulum Stress caused by Ferroptotic Reagents. Also engaged in autophagy and the release of cathepsin B, which are both implicated in

ferroptosis induction, are lysosomes. Lysosome ROS contributes to the formation of lipid reactive oxygen species (Figure 2.2) (Xu *et al.* 2019).

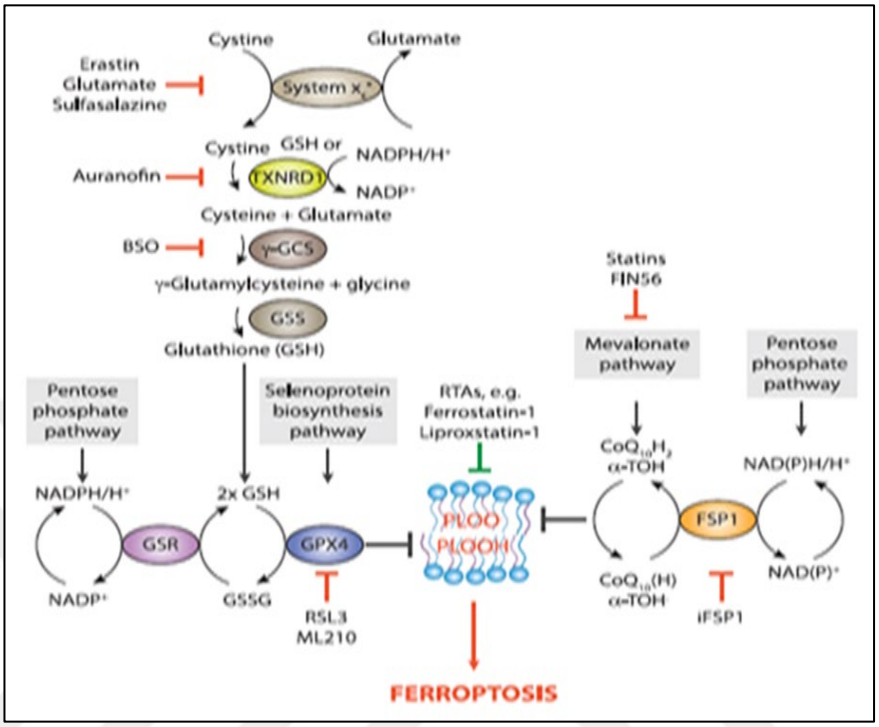

Figure 2.2 Mechanisms of ferroptosis (Conrad and Proneth 2020)

2.4 Oxidative stress

When the body's ability to prevent or detoxify free radical damage is insufficient, oxidative stress is a typical result. Unpaired electrons in one or more oxygen-containing compounds make a free radical very reactive. Free radicals may steal electrons from cell components such as DNA, protein, or lipids in order to stabilize themselves. When the cell's atoms and molecules become unstable, they begin to seek for and steal electrons from one other, leading to an endless series of free radical reactions (Alkadi, 2020). It is divided into:

2.4.1 Reactive oxygen species (ROS)

Oxygen radicals (•OH), hydrogen peroxide (H2O2), and singlet oxygen are examples of ROS (reactive oxygen species) that are produced during aerobic metabolism (O_2). Mitochondrial metabolism and NADPH oxidase at the cell membrane are the primary sources of ROS generation. The mitochondrial electron transport chain generates O_2•–, and the mitochondrial transmembrane potential governs its pace. Oxidative stress may be reduced by the presence of mitochondrial superoxide dismutase, which turns O2•– into H_2O_2. H_2O_2 creates very reactive O2•– radicals at high iron concentrations, facilitating the Fenton reaction (Costa *et al.* 2021). Due to the Haber Weiss reaction, superoxide radical is very reactive with transition metal complexes including Cu, Mn, and Fe, as shown in Reaction 2.1.

$Fe^{3+} + O_2\text{-} \rightarrow Fe^{2+} + O_2$ Haber-Weiss reaction (2.1)

Because of the more severe effects on soluble metalloproteins such [Fe-S] proteins, it seems that oxygen radical O_2•- has on superoxide anion as shown in Reaction 2.2, 2.3.

$[4Fe\text{-}4S]^{2+} + O2\text{•-} \rightarrow [4Fe\text{-}4S]\text{-}OO$ Inactivation [Fe-S] cluster (2.2)

$[4Fe\text{-}4S]\text{-}OO\text{•} + 2H^+ \rightarrow [3Fe\text{-}4S] + H_2O_2 + Fe^{3+}$ (2.3)

Nevertheless, superoxide dismutases convert O_2•- to hydrogen peroxide H2O2 (superoxide dismutase). A Fenton-type reaction with reduced cations of transition metals like Fe^{2+} or Cu^+ may cause H_2O_2 to produce hydroxyl radicals (OH•-) and hydroxyl anion (HO-), as shown in Reaction 2.4, making it a reactive oxygen species (ROS) (Rapportrice, 2016).

$Fe^{2+}(aq) + H_2O_2 \rightarrow Fe^{3+}(aq) + OH^- (aq) + OH\text{•-}$ Fenton reaction (2.4)

2.4.2 Reactive nitrogen species (RNS)

Nitric oxide synthases (NOS) primarily produce NO by converting L-arginine to NO and L-citrulline. NO is physiological effects are dependent on its local concentrations, it has a variety of functions in the nervous system and glial-regulated pathways. It aids in the regulation of neuron growth, survival, and differentiation under physiological settings. Synaptic activity, brain plasticity, and cognitive function are all influenced by nitric oxide (i.e., memory). Deficiencies in nitric oxide metabolism may lead to neuronal cell death and neurodegenerative diseases (such as Alzheimer's and Parkinson's). ROS, particularly superoxide anion, increase NO generation, which results in highly reactive peroxynitrite during neuroinflammation (ONOO-). There are transition metal sites in proteins where peroxynitrite reacts directly with them. By oxidizing ferrous heme to ferric forms, it may change proteins including hemoglobin, myoglobin, and cytochrome c. In the peptide chain, peroxynitrite may interact with multiple amino acids to alter protein structure. As nitrosative stress progresses, the most prevalent reaction is nitrotyrosination (nitrotyrosination Tyr Tyr-NO3). The covalent attachment of NO to a cysteine thiol/sulfhydryl (RSH) molecule is possible, though (S-nitrosylation RSH R-SNO). All of these processes have an effect on protein structure and function, alter enzyme catalytic performance, disrupt cytoskeletal architecture, and impair cell signaling (Cinelli *et al.* 2020).

2.5 Antioxidant System

Free radicals are unstable molecules created by the body in response to environmental and other stresses and may cause cell damage. Antioxidants may minimize or prevent this cell damage by reducing the amount of free radicals in the body. Reducing the formation of ROS, mending oxidized membranes, neutralizing free radicals and neutralizing ROS during lipid metabolism are some of the tasks that they conduct as free radical scavengers.

2.5.1 Exogenous antioxidants

As free radical scavengers, antioxidants found in food are similar to those found in supplements, but they do so in a variety of ways. A lack of naturally occurring, non-enzymatic antioxidants in the human body demands the need of synthetically synthesized antioxidants. Exogenous non-enzymatic antioxidants are categorized into three types: mineral elements, for example (selenium, Zinc, Manganese, Copper) (Bizerea *et al.* 2018). Carotenoids, vitamin E, and vitamin C are examples of phytochemicals / phytonutrients, which are naturally occurring antioxidants (Kuang *et al.* 2020). Organic peroxide, hydrogen peroxide, superoxide, and oxy radicals may be converted to water, oxygen, and alcohol by supplementation with antioxidants. Coenzyme Q10, lipoic acid, phytoestrogens and polyphenols are examples of these nutrients (Lim and Wang 2020).

2.5.2 Endogenous antioxidants

Our bodies produce endogenous antioxidants. Endogenous antioxidants are significantly more effective than exogenous antioxidants since they are created by our bodies rather than taken from dietary sources. Endogenous antioxidants heal all free radical damage by beginning cell regeneration from the inside out. Endogenous antioxidants are metabolic products of the body that may be non-enzymatic (urea, melatonin, ferritin, bilirubin, and glutathione) or enzymatic (SODs, glutathione peroxidase (GPX), glutathione reductase (GR), and catalase) or enzymatic (Pisoschi *et al.* 2021).

2.5.3 Superoxide dismutase

Superoxide radicals (O_2) are dismutated by metalloenzymes called SODs into hydrogen peroxide (H_2O_2) and oxygen (O_2). Predicted roles for SODs in the therapy of oxidative stress-related illnesses include their involvement as the first line of defense against ROS-mediated damage. In addition to being located in the cytosol and mitochondria, SODs are multimeric metaloenzymes. Cu-Zn-SOD, Fe-SOD, and Ni-SOD are only a

few of the numerous enzyme families that make up this complex. Eukaryotic cells include Cu-Zn-SOD in their chloroplasts and cytoplasms, whereas bacteria contain MnSOD in their mitochondrial matrix and cytosol and plants contain FeSOD (Ali *et al.* 2020).

2.5.4 Glutathione peroxidase (GPX)

The oxidant in a typical class I peroxidase catalytic cycle is H2O2 or organic hydroperoxide, whereas the electron donor is the tripeptide GSH. As part of the catalytic cycle that transforms selenocysteine from selenol to selenenic acid, H2O2 or ROOH are also oxidized. GSH molecules go on to create selenenyl sulfide intermediates at this point (Enz-Se-SG). After a GSH molecule assaults the oxidized and dimerized form of the enzyme Enz-Se-SG, it is released and restored to the enzyme's resting state, Enz-SeH (Sharifi-Rad *et al.* 2020).

2.5.5 Glutathione reductase (GR)

Glutathione disulfide (GSSG) is converted to glutathione by GR, a homodimer (GSH). Cellular redox balance is dependent on the presence of GSSG and GSH. glutathione S-transferase and glutathione peroxidase both rely on GSH for the detoxification of reactive oxygen species and the removal of electrophilic xenobiotics (Onyibe *et al.* 2021).

2.5.6 Catalase (CAT)

Catalase is a critical antioxidant enzyme (EC 1.11.1.6). The vast majority of aerobic organisms include it. Catalase converts two molecules of hydrogen peroxide into one oxygen molecule and two water molecules in a two-step process, as shown in Reaction 2.5.

$$H_2O_2 \text{ (Substrate) catalase/enzyme} \rightarrow 2H_2O \text{ (Water)} + O_2 \text{ (Oxygen)} \qquad (2.5)$$

There are three varieties of catalase based on changes in their sequence and structure. The most common is the monofunctional heme-containing enzyme. It's present in every aerobic creature. The second type, which is less prevalent in nature, includes the bifunctional catalase-peroxidase enzyme. This enzyme possesses a heme group as well. Plant peroxidases are structurally and functionally identical. Mn-containing catalases without the heme group belong to the third class (Nandi *et al.* 2019).

2.6 Enzyme Structure

The ferrous protoporphyrin IX prosthetic group in human heme-containing catalase interacts with hydrogen peroxide in a typical monofunctional fashion. The enzyme, which has a molecular mass of 220-240 kDa, is found in peroxisomes. In this protein, all four domains are found in the N- and C-terminal threading arms, as well as the wrapping loop and β-barrel (Figure 2.3). Eight stranded barrels surrounded by α-helices form the hydrophobic core of each subunit. Running counterclockwise, this set of β-barrels is The first four barrel domain strands (1β -4β) make up the heme distal side of the subunit, whereas the final four barrel domain strands (5β -8β) make up the NADPH binding pocket. Using a lengthy wrapping loop, the N-terminal threading arm (residues 5-70) of one subunit attaches two other subunits securely (residues 380-438). On the other side of the β-barrel, there are four C-terminal helices that form a helical domain (Nandi *et al.* 2019).

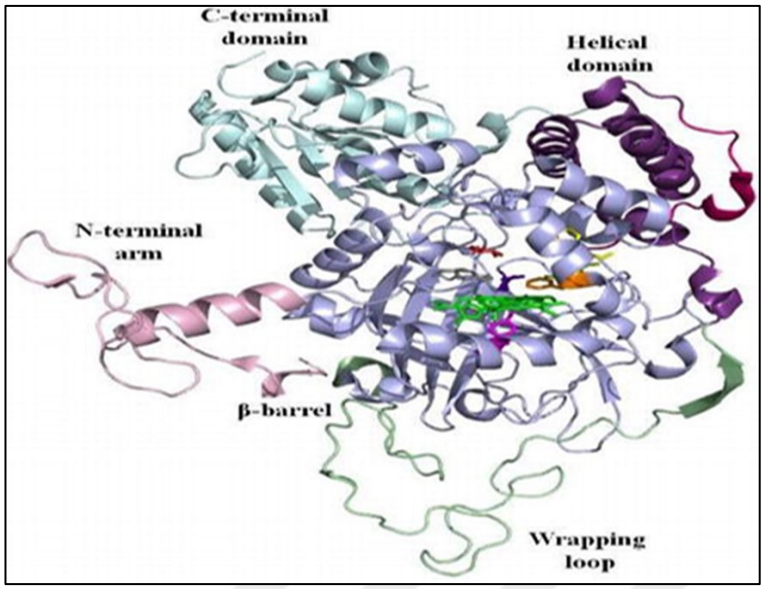

Figure 2.3 The structure of the enzyme catalase

2.6.1 Mechanism of the enzyme catalase

Catalase activity occurs in two stages. Catalase's reaction cycle is kicked off by an intermediate created by interacting high spin ferric (Fe III) with peroxide to form the compound I intermediate (a porphyrin -cation radical containing Fe IV). They are exchanging one of the hydrogen peroxide molecule's protons. The proton is transported via a histidine residue at the active site. Hydrogen peroxide's O-O bond is polarized and broken by this mechanism. The enzyme is then reactivated by adding a second molecule of hydrogen peroxide to act as a reductant, resulting in the generation of water and oxygen. A rapidly oxidizing Fe(IV) molecule, as shown in Reaction 2.6, 2.7, compound I, is converted to its native resting state Fe by the oxidation of an electron donor (in this instance, second H2O2) (III) (Mehrotra, 2020).

$$H_2O_2 + \text{Fe (III)-enzyme} \rightarrow H_2O + \text{O = Fe(IV)- enzyme (compound I)} \quad (2.6)$$

$$H_2O_2 + \text{O = Fe(IV)- enzyme (compound I)} \rightarrow H_2O + O_2 + \text{Fe (III)-enzyme} \quad (2.7)$$

3. MATERIALS AND METHODS

3.1 Chemicals and Reagent

All devices and chemicals were listed in this study, as shown in Table 3.1.

Table 3.1 The chemicals and reagent used in this study

NO.	CHEMICALS	PURITY %	SUPPLIED COMPANY
1	guanidine hydrochloride		BDH
2	2,4-dinitrophenyl hydrazine	99	BDH
3	2,9-dimethyl-1,10-phenanthroline(Neocuproine). $C_{14}H_{12}N_2$	98	Sigma-Aldrich
4	Ammonium acetate.(NH_4Ac).	99	BDH
5	Ammonium metavanadate	99	BDH
6	Potassium dihydrogen phosphate KH_2PO_4		BDH
7	Disodium hydrogen phosphate Na_2HPO_4. $2H_2O$		BDH
8	5,5'-Dithio-bis-(2-nitrobenzoic acid) (DTNB)		
9	Copper (II) chloride $CuCL_2.2H_2O$	99	Sigma-Aldrich
10	Ethanol	99	
11	Ferritin kit (Lot: 1009051820)		Elab-science
12	Ferrous ammonium sulfate $Fe(SO_4)(NH4)_2(SO_4)$	99	BDH
13	glutathione (GSH)		Sigma-Aldrich
14	Glutathione peroxidase 4 Elisa kit		Elab-science
15	Glycerol ($C_3H_8O_3$)	99	BDH
16	Uric acid	99	BDH
17	Hexan	98	BDH
18	Hydrochloric acid (HCl)	99	BDH
19	Hydrogen peroxide (H_2O_2)		Fluka
20	Xylenol orange ($C_{31}H_{32}N_2O_{13}S$)	99	BDH
21	O-dianisidine dihydrochloride $C_{14}H_{16}N_2O_2.2HCl$	99	Sigma-Aldrich
22	Sodium chloride (NaCl)	99	BDH
23	Ethyl acetate	99	BDH
24	Sodium hydroxide (NaOH)	99	BDH
25	Sulfuric acid (H_2SO_4)	98	BDH
26	Thiobarbituric acid. (TBA). $C_4H_4N_2O_2S$	99	Sigma-Aldrich
27	Trichloroacetatic acid. (TCA). $C_2HCl_3O_2$	99	BDH

3.2 Instrument Used

All instrument were listed in this study, as shown in Table 3.2.

Table 3.2 Instrument used

NO.	INSTRUMENT	SUPPLIED COMPANY
1	Centrifuge	Heraeus (Germany)
2	Deep Freez	GFL / Germany
3	Magnetic stirrer	Gallin kamp (England)
4	Micro-centrifuge	Hettich / Germany
5	Microplate reader	BioTek (USA)
6	Oven	Hearson (England)
7	PG T80+ Spectrophotometer	Shimadzu / (USA)
8	pH meter	Jenway (Germany)
9	Sensitive balance	Stanton 461 AN (Germany)
10	Spectrophotometer UV -1601	Shimadzu / (USA)
11	Vortex mixer	Karlkole (Germany)
12	Water bath	Karlkole (Germany)
13	VIDAS	France
14	Auto Hematology Analyzer	Germany

3.3 Methods

3.3.1 Sampling

In our study overall 300 male subjects were choosing (150 patients with β-thalassemia major, 150 healthy control persons). All participants were aged (5-15 years) via measuring:

3.3.2 Measure of blood parameters that relate with β-thalassemia, such as HbA and ferritin.

VIDAS Ferritin kit (batch number 1009051820) was used to determine serum ferritin concentration. also The Auto Hematology Analyzer (Model:BC-10) (LOT:2021081101)

Complete System was used to determine the Hemoglobin levels of children at the Central Blood Laboratory - Thalassemia Center in Babylon.

3.3.3 Measure catalase enzyme activity

Principle: Vanadium (V) is reduced to vanadium (III) by H2O2 in the process. H2O2 is a powerful oxidant that may also operate as a reductant under specific redox circumstances. Consequently, when vanadium (V) is reduced, a red–orange peroxovanadium complex, with a maximum absorbance of 450nm, is created. The following equation shows the reaction between vanadium and H_2O_2, as shown in Reaction 3.1:

$$H_2O_2 + NH_4VO_3 + H_2SO_4 \rightarrow NH_4[VO(O_2)SO_4] + 2H_2O \quad (3.1)$$

3.3.4 Assessment total oxidant status

principle: A technique devised by Erel was used to determine serum TOS. O-dianisidine and ferrous ion in the serum react to form ferric ion. In the presence of glycerol molecules, the oxidation process may be accelerated. Fe3+ and xylenic orange combine in acidic environments to produce an attractive hue. In order to measure the color intensity of a sample, spectrophotometrically, the total number of oxidant molecules in the sample may be calculated. mol H2O2 Eq/L is the standard unit of measurement for these data, which were calibrated using hydrogen peroxide as a calibration standard.

3.3.5 Assessment total antioxidant capacity the CUPRAC method

Principle, as shown in Reaction 3.2, 3.3:

$$\text{Antioxidants} + Cu^{+2} \rightarrow Cu^{+2} \quad (3.2)$$

$$Cu^{+}\text{+2,9-dimethyl-1, 10-phenanthroline} \rightarrow \text{complex } (\lambda \text{ max at 450 nm}) \quad (3.3)$$

3.3.6 Measure of lipid peroxidation

Principle: The Thio-burbiturate reacting substance (TBARS) technique was used to test serum malondialdehyde (MDA). Damage-causing free radical-driven auto-oxidation happens when polyunsaturated fatty acids and molecular oxygen come together, resulting in a large number of aldehyde molecules and numerous peroxides. These non-volatile molecules are broken down into TBARS and other compounds in the laboratory by acid heating. When TBARS and thio-barbituric acid are combined in an acidic environment, the result is a pink-colored compound. After extraction in organic solvents like butanol, the absorbance of this molecule is measured at 532 nm, as shown in Figure 3.1.

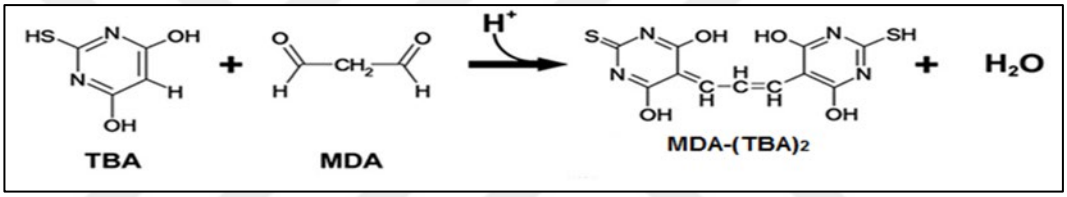

Figure 3.1 Scheme of the adduct MDA–$(TBA)_2$

3.3.7 Measure of protein carbonyl group

Principle: 2,4-dinitrophanylhydrazine is illustrated in the as combining with carbonyl groups to create a hydrozone derivative (Figure 3.2). With 20 percent trichloroacetic acid and DNPH, protein precipitation occurred immediately. It was then established that the carbonyl content of the samples was evaluated using a 370 nm absorbance test.

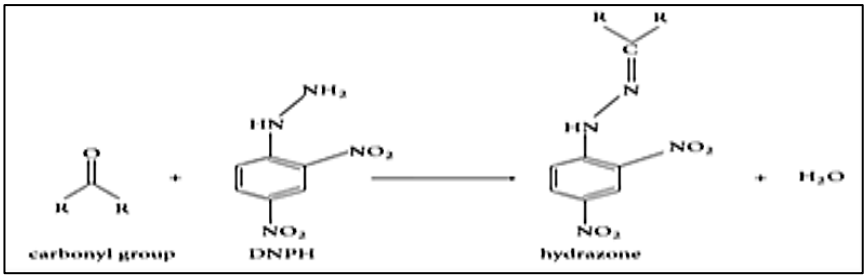

Figure 3.2 Precipitation of the carbonyl group

3.3.8 Assess total thiol concentration

Principle: One of the free sulfuryl groups reacts with 5,5'Dithio-bis-(2-nitrobenzoic acid) to create a mixed disulfide and 2-nitro-5-thiobenzic acid. A free sulfhydryl group's conjugate base (R—S-) serves as a target for DTNB (Figure 3.3). This reaction produces 2-nitro-5-thiobenzoic acid, which has a high molar extinction coefficient in the visual range and is the "hued" product. Originally, it was stated that 2-nitro-5-thiobenzoic acid's molar extinction coefficient at 405 nm and pH 8 was 13,600 $M^{-1}cm^{-1}$.

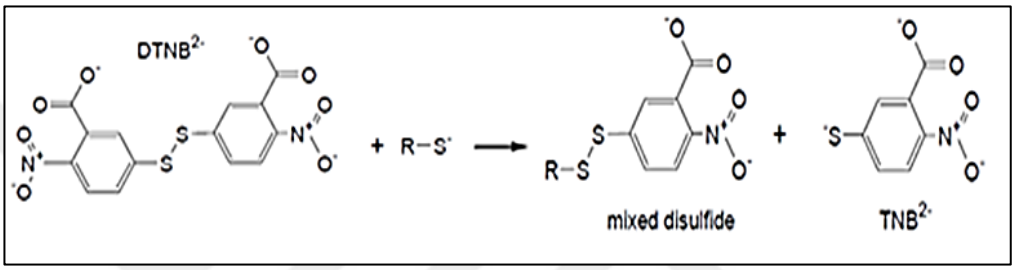

Figure 3.3 Reaction of ellman's reagent with thiols

3.3.9 Measure glutathione peroxidase 4 concentration (E6887Hu)

Principle: The quantitative sandwich enzyme immunoassay method is used in this test. An anti-GPX4 antibody has been pre-coated on a microplate. samples and standards are added to the wells, and the antibody binds any GPX4 that may be in the samples or standards. Following incubation, unbound samples are removed during a wash phase, and a detection antibody specific for GPX4 is introduced to the wells, binding to the capture antibody-GPX4 combination in the sample. After a wash, the enzyme conjugate is injected into the wells to remove any unbound mixtures. After the incubation and washing steps, a substrate is introduced. The quantity of GPX4 in the sample causes the formation of the colored product TMB. After adding acid to stop the reaction, the absorbance is measured. Seven GPX4 standard dilutions are used to create a standard curve, and the GPX4 sample concentration is calculated.

4. RESULTS AND DISCUSSION

4.1 Biochemical and Clinical Characteristics of the Subject of the Study

The current study included (300) children divided into two groups, the first (150) healthy children who did not suffer from any health problems and who worked as a control group in the study. As for the second group (150) patients with Beta-Thalassemia major who are treated for clinical symptoms and disease manifestations in Babel Teaching Hospital for Maternity and Children in Iraq. The controls were (5-15) years old and males in both groups, and the results are presented in the form of tables and the comparison between the values.

4.2 Biochemical and Clinical Characteristics of the Study Subjects

Table 4.1 shows that the levels of ferritin (3337.0 ± 1475) in the blood of beta-thalassemia major patients are much higher than the levels of ferritin (44.5 ± 25.15) in healthy controls, which is consistent with what was stated in (Sengsuk *et al.* 2014). Ferritin is the body's main iron storage protein.And that the ferritin indices increased significantly in Beta- Thalassemia major the blood, which increased more than in the healthy controls. Iron overload occurs in beta-thalassemia major via two primary mechanisms: Due to a lack of erythropoiesis and recurrent blood transfusions, iron absorption is enhanced. O2 (superoxide anions) and other reactive oxygen species (OH•, monooxygen and hydrogen peroxide) are produced in people with thalassemia major, which causes oxidative stress via the Fenton reaction.The hemoglobin value was in beta- thalassemia major patients (7.5±1.0) and in the control subjects (11.5±0.75). The findings demonstrated that beta-thalassemia major patients had lower hemoglobin levels than control participants, which is consistent with what was previously stated (Origa, 2017). The basic cause of β-thalassemia is an imbalance in the -/non-globin chains. In erythroid precursors, aggregation and precipitation of Globin tetramers leads to apoptosis and oxidative membrane damage. RBC precursors in the bone marrow are severely depleted when these inclusion bodies are linked to the membrane skeleton (ineffective Red blood cells).

Table 4.1 General and clinical characteristics of the study groups

CHARACTER	PATIENTS	CONTROLS	P-VALUE
Age	9.5 ± 2.2	9.2 ± 1.29	0.91
Ferritin	3337.0±1475	44.5±25.15	< 0.001
Hb (g /dL)	7.5±1.0	11.5±0.75	<0.01

4.3 Catalase Activity

The enzyme activity of catalase was estimated in the blood serum of patients with Beta-thalassemia major. It was found that the enzyme activity rate (0.2716) as well as the catalase enzyme activity rate in the blood serum of the control group (0.2202) as shown in the Table 4.2. The serum activity of Beta-thalassemia major patients was shown to be much higher than the activity of the control enzyme, which is compatible with what was stated by the researchers (Qaiser *et al.* 2015). In individuals with beta thalassemia major, peroxidative damage caused by secondary iron overload is the predominant source of oxidative stress. Beta thalassemia major patients have lower levels of reduced glutathione, which is the body's first line of defense against free radicals and the source of tissue damage. In the event of oxidative stress, increased CAT activity may serve as a protective mechanism.

Table 4.2 Catalase activity (Katal/mL) in beta-thalassemia major patients' sera compared to healthy controls

GROUP	N.	MEAN	STD. DEVIATION	STD. ERROR	SIGN.
Control	150	0.2202	0.14394	0.011	---
Patients	150	0.2716	0.20937	0.017	0.014*
*: significance versus group I (Healthy donors)					

4.4 Total Oxidant (ROS) and Total Antioxidant (TAC)

In this method, the total oxidative stress status (ROS) was estimated in patients and healthy observers, and we observed in Table 4.3 the appearance of a significant increase

in ROS values in thalassemia patients (5.5559) compared to ROS values in the control group (4.4078) and this is in agreement with what was mentioned (Ghone *et al.* 2008). Unpaired alpha-globin chains accumulate when there aren't enough beta-globin chains. In addition to iron overload, extra alpha-globin chains are a major contributor to cellular oxidative damage. Beta-thalassemia is associated with greater levels of plasma iron and intracellular non-hemoglobin iron, both of which promote the formation of reactive oxygen species (ROS). In such a situation, the body's own antioxidants are likely to decline.

Table 4.3 Serum total oxidant (mol/L) in beta thalassemia major patients compared to healthy controls

GROUP	N.	MEAN	STD. DEVIATION	STD. ERROR	SIGN.
Control	150	4.4078	1.74168	0.14221	---
Patients	150	5.5559	1.87697	0.15325	0.001*
*: significance versus group I (Healthy donors)					

Total antioxidant levels (700.9567 vs. 951.6667) were significantly lower in thalassemia major patients as reported in Table 4.4 and this is consistent with what was observed (HANAN *et al.* 2020) Beta thalassemia major patients' enhanced hemolysis may be linked to iron overload and an increase in ROS generation. Antioxidants are important components of the body's defensive system because they protect it from free radicals or help it break them down. which may be attributed to their consumption for neutralizing the impact of excess ROS and oxidative stress.

Table 4.4 Total antioxidant (mol/L) in beta-thalassemia major patients' sera compared to healthy controls

GROUP	N.	MEAN	STD. DEVIATION	STD. ERROR	SIGN.
Control	150	951.6667	103.72535	8.46914	---
Patients	150	700.9567	84.35541	6.88759	0.001*
*: significance versus group I (Healthy donors)					

4.5 Malondialdehyde (MDA)

As shown in Table 4.5 the mean MDA was significantly higher in the main beta - thalassemia major patients, which is (2.8624) compared to the healthy ones (3.7844) indicating increased oxidative stress and this is consistent with what was found in (Youssef *et al.* 2019). Malondialdehyde concentrations in beta-thalassemia major patients are increased, resulting in increased oxidative stress and lipid oxidation, which produces a variety of chemicals, including MDA. As part of the DNA-MDA coupler, malondialdehyde plays an important role in carcinogenesis. In many cancers, DNA-MDA techniques generate mutations in a number of genes, including tumor suppressor genes and oncogenes, and cell cycle changes. Damage to the polyunsaturated fatty acid-rich red blood cell membranes has been associated to elevated levels of lipid peroxidation and MDA. A spike in free radical levels is associated with an increase in lipid peroxidation in numerous types of cancer. Oxidative stress, on the other hand, is well-detected by MDA. The amount of free oxygen radicals generated and not eliminated by the body's defense system may be determined by measuring the degree of lipid oxidation. Oxidative stress seems to be a significant risk factor. Due to repeated transfusions in these people, secondary iron overload causes oxidative tissue damage. Unpaired alpha-globin chains accumulate when there aren't enough beta-globin chains. In addition to iron overload, extra alpha-globin chains are a major contributor to cellular oxidative damage. Beta-thalassemia major is characterized by elevated levels of plasma iron and intracellular non-hemoglobin iron. Furthermore, repeated blood transfusions cause iron excess, which enhances the generation of free radicals and tissue oxidation damage. Endogenous antioxidants are expected to be reduced in such a condition.

Table 4.5 Malondialdehyde (mol/L) in the sera of beta-thalassemia major patients compared to healthy controls

GROUP	N.	MEAN	STD. DEVIATION	STD. ERROR	SIGN.
Control	150	2.8624	0.87980	0.07184	---
Patients	150	3.7844	1.06917	0.08730	0.025*
*: significance versus group I (Healthy donors)					

4.6 Total Thiol

For the Thiols group test, the mean values were in the thalassemia group (381.8922), while the averages were (417.1471) in the control group, there was a significant decrease in the values of beta-thalassemia major patients compared to the values in the control group. As shown in Table 4.6 and this is consistent with what you mentioned for (Lamia, 2007). The thiol group contains a possible sulfhydryl group (SH) in its structure and may be found in the amino acids cysteine, methionine, and protein. The oxidative stress in patients and the sulfhydryl group in proteins being oxidized as a result of excessive generation of free radicals like superoxide anion are further examples of oxidative stress (O2-). Thyol groups have also been demonstrated to be oxidatively degraded in serum and are often low in people with thalassemia, hence, thiol group screening may help identify illness concerns.

Table 4.6 Total thiol (mol/L) in sera of beta-thalassemia major patients vs healthy controls

GROUP	N.	MEAN	STD. DEVIATION	STD. ERROR	SIGN.
Control	150	417.1471	160.21734	13.08169	---
Patients	150	381.8922	90.87928	7.42026	0.017*
*: significance versus group I (Healthy donors)					

4.7 Total Carbonyl Group

The results shown in the Table 4.7 showed that the average values of total protein carbonyl in patients with Beta- thalassemia major (1.3975) were significantly higher than the average values of the control group (1.0893) and this corresponds to what was mentioned (Trombetta *et al.* 2006). It has been shown that the carbonyl concentration in blood protein, an indicator of oxidative stress, increases with several diseases and processes. Beta thalassemia patients had greater amounts of protein carbonyl groups in their blood, according to a new study. High quantities of free heme, which occur during bouts of acute hemolysis, may induce toxicity, resulting in organ, tissue, and cellular

damage. Free heme generates ROS, which damages lipids, proteins, and DNA, but it also catalyzes oxidation, covalent binding, complete protein synthesis, and its breakdown into tiny peptides. The presence of protein clusters correlates strongly with illness development and severity in various disease conditions.

Table 4.7 Serum total carbonyl group (nmol/mg protein) in beta-thalassemia major patients compared to healthy controls

GROUP	N.	MEAN	STD. DEVIATION	STD. ERROR	SIGN.
Control	150	1.0893	0.23592	0.01926	---
Patients	150	1.3975	0.25089	0.02048	0.017*
*: significance versus group I (Healthy donors)					

4.8 Glutathione Peroxidase 4 (GPX4)

The results that we obtained in Table 4.8 showed a significant decrease in GPX4 activity rate (5.3741) for Beta- thalassemia major patients compared to GPX4 values (7.8095) for the control group and this is consistent with what was mentioned (Lei 2019, Hu *et al.* 2019). When it comes to the conversion of hazardous lipid hydroperoxides found in biological membranes, only one enzyme may employ glutathione as an electron donor: Glutathione peroxidase 4. The iron-dependent cell death process known as non-apoptotic ferroptosis has been related to a variety of neurological illnesses that are characterized by high levels of oxidative stress. In ferroptosis, a kind of nonapoptotic controlled cell death, iron-dependent lipid peroxide is formed. In terms of structure and biology, it is separate from other cell death. The therapy of cancer, renal failure, and ischemia reperfusion damage all need ferroptosis. Cysteine deficiency and arachidonoyl peroxidation are the primary causes of ferroptosis, but glutathione peroxidase 4 (GPX4) suppression is also a contributing factor. Antioxidants, iron chelators, and an inhibitor all have a role in preventing ferroptosis. It is possible to utilize iron chelators and lipid peroxidation inhibitors to reduce the development of lipid peroxidation products caused by iron-catalyzed polyunsaturated fatty acid oxidation in order to limit ferroptosis biochemically. Glutathione (GSH), an important cofactor for

the enzyme, converts gas-phase peroxyl radicals to harmless lipid alcohols. Glutathione peroxidase 4 (GPX4) protects cells against ferroptosis by removing internal lipid ROS, while inhibiting GPX4 causes ferroptosis. There are eight nucleophilic amino acids in Glutathione peroxidase 4 that might react with electrophiles in the cell (one selenocysteine and seven cysteines). Since cysteine may make cells more ferroptosis-sensitive, selenium is required for GPX4 to maintain its ferroptosis-resistance function. Fatal lipid peroxide buildup is considered to be caused by the deactivation or absence of GPX4. A major stage in ferroptosis is the inhibition of GPX4.

Table 4.8 Glutathione peroxidase 4 (ng/mL) in beta-thalassemia major patients' sera compared to healthy controls

GROUP	N.	MEAN	STD. DEVIATION	STD. ERROR	SIGN.
Control	150	7.8095	5.22576	0.42668	---
Patients	150	5.3741	3.08044	0.25152	0.01*
*: significance versus group I (Healthy donors)					

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

1. Ferritin levels were significantly greater in beta-thalassemia major patients than in healthy controls.
2. Hemoglobin levels in beta-thalassemia major patients were much lower than in healthy individuals.
3. Catalase Patients with beta-thalassemia major were more active than the general population.
4. Compared to healthy persons, beta-thalassemia major patients had considerably greater levels of total oxidant (ROS) in their bodies.
5. TAC levels were substantially lower in beta-thalassemia major patients than in healthy controls.
6. In beta-thalassemia major patients, lipid oxidation (Malondialdehyde (MDA)) levels were much higher than in healthy people.
7. In beta-thalassemia major patients, the total thiol group level was much lower than in healthy people.
8. In beta-thalassemia major patients, the total carbonyl group level was much higher than in healthy people.
9. Beta-thalassemia major patients have reduced levels of glutathione peroxidase 4 (GPX4) compared to healthy individuals.
10. Due to a substantial drop in Total thiol group levels, Glutathione peroxidase 4 (GPX4) levels, and an increase in lipid oxidation (Malondialdehyde (MDA) levels and ferritin levels, the present investigation found the existence of the ferroptosis illness.

5.2 Recommendations

1. Further study of the relationship between thalassemia and ferroptosis.
2. Educate people to have full testing to detect and avoid people who carry the thalassemia genes.

3. Advise patients to pay attention to their diets by increasing antioxidant-rich foods and decreasing oxidant-rich foods.

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